AD-A197 720 ; DOCUMENTATION PAGE Page Approved Green Approved Gr											[4]
Inclassified 25 SECURIT CLASSIFICATION AUTOPT 26 DECLASSIFICATION AUTOPT 27 DECLASSIFICATION REPORT NUMBERGY 28 PROFESSIONAL OR PROFESSIONAL STREET A PERFORMING ORGANIZATION REPORT NUMBERGY BE NAME OF PRODUCTIONS DISCONSET SCREEN SCREEN TO U.S. ALTRY Medical Research and Development of DESCRIPTION OF ADDRESS (CR), State, and ZPCOOD) FORT Detrick, Frederick, MD 21701-5011 BE NAME OF PUNDING PONSORING ORGANIZATION (PROPERTY OF ADDRESS (CR), State, and ZPCOOD) FORT Detrick, Frederick, MD 21701-5011 BE NAME OF PUNDING PONSORING ORGANIZATION NUMBER (PROPERTY OF ADDRESS (CR), State, and ZPCOOD) BE NAME OF PUNDING PONSORING ORGANIZATION NUMBER (PROPERTY OF ADDRESS (CR), State, and ZPCOOD) BE NAME OF PUNDING PONSORING ORGANIZATION NUMBER (PROPERTY OF ADDRESS (CR), State, and ZPCOOD) BE NAME OF PUNDING PONSORING ORGANIZATION NUMBER (PROPERTY OF ADDRESS (CR), State, and ZPCOOD) BE NAME OF PUNDING PONSORING ORGANIZATION NUMBER (PROPERTY OF ADDRESS (CR), State, and ZPCOOD) BE NAME OF PUNDING PONSORING ORGANIZATION NUMBER (PROPERTY OF ADDRESS (CR), State, and ZPCOOD) BE PROCURED NOT THE STATE OF ADDRESS (CR), State, and ZPCOOD) BE PROCURED NOT THE STATE OF ADDRESS (CR), STATE, A	AD-	-A 19	7 7	720 _[DOCUMENTATI	ON PAG	E				
1. Diffestion animals 1. D			31F1CM 1 i	ion k T	2 1 Break	16 REST	RICTIVE	MARKINGS ;	1-1-	1	
Distribution unlimited - approved for public release 4 PERFORMING ORGANIZATION REPORT NUMBERS) 5 MONIFORMING ORGANIZATION REPORT NUMBERS 5 MONIFORMING ORGANIZATION REPORT NUMBERS 5 MONIFORMING ORGANIZATION REPORT NUMBERS) 5 MONIFORMING ORGANIZATION REPORT NUMBERS 6 ADDRESS (City, State, and JIP Code) 5 MONIFORMING ORGANIZATION REPORT NUMBERS 6 ADDRESS (City, State, and JIP Code) 5 MONIFORMING ORGANIZATION REPORT NUMBERS 6 MONIFORMING ORGANIZATION REPORT NUMBERS FOR ART AND ALL THE ALL THE AND ALL THE ALL THE ALL THE AND ALL THE ALL T			ON AUT	HORRY	-ESTERN	3 DISTE	RIBUTION	/AVAILABILITY	OF REPORT		
4. PERFORMING ORGANIZATION REPORT NUMBER(S) 5. MONE ORING ORGANIZATION REPORT NUMBER(S) 6. ADMESS CONT. STATES AND					6-1-19-6-1-1-	Distr	ibuti	on unlimit			for
Se NAME OF PERFORMING GRGANIZATION (a., Army Medical Research Inditition of Infortions Diseases SGRD-UIS-D Sc ADDRESS (CRy, State, and IP Code) SGRD-UIS-D Fort Detrick, Frederick, MD 21701-5011 Fort Detrick, Frederick, MD 21701-5012	26. DECLASSI	FICATION/DOS	WNGRA	DING ACHEDI		publi	c rel	eas e			
U.S. Army Medical Research and Development	4. PERFORMI	ng Organiza	TION RE	PORT NUMB	D 9	5. MONI	TORING	ORGANIZATION	REPORT N	UMBER(S)	
Intitute of Infections Diseases SGRD-UIS-D Command	6. NAME OF	PERFORMING	GRGAN	IZATION		7a NAN	E OF M	ONITORING ORG	ANIZATION		
FORT Detrick, Frederick, MD 21701-5011 Ea. NAME OF FUNDING/SPONSORING ORGANIZATION BC. ADDRESS (City, State, and ZiP Code) BC. ADDRESS (City, State, and ZiP Code) BC. ADDRESS (City, State, and ZiP Code) 10. School Coff Funding Numbers PROGRAM POLECT TASK ACCESSION 11. Iffice (include Security Classification) Immunobletection of T-2 Metabolities in Rat Urines After Dermal, Oral, or Intramiscular Exposure to T-2 Toxin 17. PERSONAL AUTHOR(S) 19. The Covered To Toxin 18. Subject TERMS (Continue on reverse in necessary and dentify by Block number). 19. COSATI CODES 18. Subject TERMS (Continue on reverse in necessary and dentify by Block number). 19. ABSTRACT (Continue on reverse in necessary and dentify by Block number). 19. ABSTRACT (Continue on reverse in necessary and dentify by Block number). 19. ABSTRACT (Continue on reverse in necessary and dentify by Block number). 19. ABSTRACT (Continue on reverse in necessary and dentify by Block number). 19. ABSTRACT (Continue on reverse in necessary and dentify by Block number). 19. ABSTRACT (Continue on reverse in necessary and dentify by Block number). 19. ABSTRACT (Continue on reverse in necessary and dentify by Block number). 19. ABSTRACT (Continue on reverse in necessary and dentify by Block number). 19. ABSTRACT (Continue on reverse in necessary and dentify by Block number). 19. ABSTRACT (Continue on reverse in necessary and dentify by Block number). 19. ABSTRACT (Continue on reverse in necessary and dentify by Block number). 19. ABSTRACT (Continue on reverse in necessary and dentify by Block number). 19. ABSTRACT (Continue on reverse in necessary and dentify by Block number). 19. ABSTRACT (Continue on reverse in necessary and dentify by Block number). 19. ABSTRACT (Continue on reverse in necessary and dentify by Block number). 19. ABSTRACT (Continue on reverse in necessary and dentify by Block number). 19. ABSTRACT (Continue on reverse in necessary and dentify by Block number). 20. The number of necessary (RIA) with an encryman and pro						1		Medical Res	search .	and De	velopment
FORT Detrick, Frederick, MD 21701-5012 8a. NAME OF FUNDING/SPONSORING (# approaches) 8b. ADDRESS (Gty. State, and ZIP Code) 8c. ADDRESS (Gty. State, and ZIP Code)					g SGRD-UIS-D		1 .	v Cease and St	O Conda		
BE. NAME OF FUNDING/SPONSORING BE. ADDRESS (City, State, and ZiP Code) BE. A	OC. ADDRESS	(City, state, ar	NJ 21" ((····		78. 200	/r E > 3 (C /l	y, state, and zii	r cooe)		
BC. ADDRESS (City, State, and ZiP Code) 10 SOLINGE OF FUNDING NUMBERS PROGRAY NO NO TAX ACCESSION 11 Iffice (Include Security Classification) Immunicule tection of T-2 Metabolities in Rat Urines After Exposure to T-2 Toxin 12 PERSONAL AUTHORIS) 13 INPE OF REPORT 13 TIME COVERED 14 DATE OF REPORT (Year, Month, Day) 15 PAGE COUNT 16 SUPPLEMENTARY NOTATION 17 COSAN CODES 16 SUBJECT TERMS (Continue on reverse if necessary and mentify by block number). 18 SUPPLEMENTARY NOTATION 19 ABSTRACT (Continue on reverse if necessary and mentify by block number). 19 ABSTRACT (Continue on reverse if necessary and mentify by block number). 19 ABSTRACT (Continue on reverse if necessary and mentify by block number). 19 ABSTRACT (Continue on reverse if necessary and mentify by block number). 19 ABSTRACT (Continue on reverse if necessary and mentify by block number). 10 GROUP 11 TIME SUPPLEMENTARY NOTATION 12 COSAN CODES 16 SUBJECT TERMS (Continue on reverse if necessary and mentify by block number). 17 COSAN CODES 16 SUBJECT TERMS (Continue on reverse if necessary and mentify by block number). 18 ABSTRACT (Continue on reverse if necessary and mentify by block number). 19 ABSTRACT (Continue on reverse if necessary and mentify by block number). 10 ABSTRACT (Continue on reverse if necessary and mentify by block number). 11 ABSTRACT (Continue on reverse if necessary and mentify by block number). 12 ABSTRACT (Continue on reverse if necessary and mentify by block number). 13 ABSTRACT (Continue on reverse if necessary and mentify by block number). 14 DATE OF REPORT (Year, Month, Day) 15 PAGE COUNT 16 SUBJECT TERMS (Continue on reverse if necessary and mentify by block number). 17 COSAN (CODES) 18 SUBJECT TERMS (Continue on reverse if necessary and mentify by block number). 18 ABSTRACT (Continue on reverse if necessary and mentify by block number). 19 ABSTRACT (Continue on reverse if necessary and mentify by block number). 19 ABSTRACT (Continue on reverse if necessary and mentify by block number). 19 ABSTRACT	fort Detr	ick, Fred	erick	, MD 21	701-5011	Fort	lætri	ck, Freder	ick, MD	2170	1-5012
PROGRAM NO PROJECT TASK ACCESSION Introduct Security Classification) Immunoletection of T-2 Metabolites in Rat Urines After Exposure to T-2 Toxin 12 PERSONAL AUTHOR(S) 13-IPHO PR REPORT 14 DATE OF REPORT (Year, Month, Dey) 15 PAGE COUNT 16 SUPPLEMENTARY NOTATION 16 SUPPLEMENTARY NOTATION 17 COSAL CODES 18 SUBJECT TERMS (Continue on reverse if necessary and mentify by block number). 19 ABSTRACT (Continue on reverse if necessary and mentify by block number). 19 ABSTRACT (Continue on reverse if necessary and mentify by block number). 19 COSAL CODES 10 SUBJECT TERMS (Continue on reverse if necessary and mentify by block number). 19 ABSTRACT (Continue on reverse if necessary and mentify by block number). 19 COSAL CODES 10 SUBJECT TERMS (Continue on reverse if necessary and mentify by block number). 10 COSAL CODES 11 SUBJECT TERMS (Continue on reverse if necessary and mentify by block number). 11 COSAL CODES 12 COSAL CODES 13 SUBJECT TERMS (Continue on reverse if necessary and mentify by block number). 14 CATE OF REPORT (Year, Month, Dey) 15 PAGE COUNT 21 TASS (Continue on reverse if necessary and mentify by block number). 16 SUBJECT TERMS (Continue on reverse if necessary and mentify by block number). 17 COSAL CODES 18 SUBJECT TERMS (Continue on reverse if necessary and mentify by block number). 19 ABSTRACT (Continue on reverse if necessary and mentify by block number). 10 Tass (Continue on reverse if necessary and mentify by block number). 11 ABSTRACT SECRETY (CLASSIFICATION) 12 DASTRACT SECRETY CLASSIFICATION 13 DASTRACT SECRETY CLASSIFICATION 14 DASTRACT SECRETY CLASSIFICATION 15 TERMS (PROME MENCOW AREA CONT) 16 SUBJECT TERMS (Continue on reverse if necessary and mentify by block number). 17 COSAL CODES 18 SUBJECT TERMS (Continue on reverse if necessary and mentify by block number). 18 SUBJECT TERMS (Continue on reverse if necessary and mentify by block number). 19 ABSTRACT SECRETY (CLASSIFICATION).			DNSORIN	NG		9 PROCU	REMEN	INSTRUMENT I	DENTIFICAT	TION NUM	ABER
If fifte (include Security Classification) Immunobetection of T-2 Metabolites in Rat Urines After Exposure to T-2 Toxin 17 FERSONAL AUTHORS) 17-18 FERSONAL TO THE COVERED TO THE COUNT TO THE	Sc. ADDRESS	City, State, and	I ZIP Cod	de)		10 501 6	CE OF F	UNDING NUMBE	RS		
If the model security Classification of T-2 Metabolites in Rat Urine's After Dermal, Oral, or Intramuscular Exposure to T-2 Toxin 12 PERSONAL AUTHOR(S) 13 INFECOMEN AUTHOR(S) 13 INFECOMEN AUTHOR(S) 14 DATE OF REPORT J. Hawley 15 INFECOMEN AUTHOR(S) 16 INFECOMEN AUTHOR(S) 17 COSAN CODES 18 SUBJECT TERMS (Continue on reverse of mercessary and identify by block number). 19 ABSTRACT (Continue on reverse of mercessary and identify by block number) 19 ABSTRACT (Continue on reverse of mercessary and identify by block number) 19 Later of the animals author of the				•							MORK UNIT
Immunicate tection of T-2 Metabolites in Rat Urines After Dermal, Oral, or Intramuscular Exposure to T-2 Toxin 17 PERSONAL AUTHOR(S) 19 PERSONAL AUTHOR(S) 19 PERSONAL AUTHOR(S) 19 THE OF REPORT 135 TIME COVERD 14 DATE OF REPORT (Year, Month, Dep) 15 PAGE COUNT (Interim FROM To 25 July 1988 21 25 July 1988 21 25 July 1988 21 26 SUPPLEMENTARY NOTATION 17 COSATI CODES 18 SUBJECT TERMS (Continue on reverse if necessary and identify by block number). 18 FIELD GROUP SUBJECT TERMS (Continue on reverse if necessary and identify by block number). 19 ABSTRACT (Continue on reverse if necessary and identify by block number). 1-2 toxin metabolites were detected in rat urines up to 2 weeks after the animals¹ exposure to subject that dose of T-2 toxin. Drings were assayed for HT-2 or T-2 tetraol by / readioinmonosassay (RIA) with polyclonal antibxdy. Sensitivity of the RIA was S n) all for HT-2 and 50 ng/ml for T-2 tetraol. Some urines were assayed in parallel with an enzymeticked immunication assay (ELISA) developed for T-2 with a monoclonal antibxdy that cross reports with HT-2. The sensitivity of the ELISA for detection of HT-2 in rat urines was 25 m/ml. Hirnes were diluted 1/2 or 1/5 to eliminate high backgrounds, which appears as a rise positives in the T-2 tetraol RIA and ELISA assays. T-2 or its metabolites could be detected by the ELISA correlated with the HT-2 or HT-2-like campounds detected by RIA. 20 DISTRIBUTION/AVAILABILITY OF ABSTRACT One USERS 21 ABSTRACT SECONITY CLASSIFICATION 12 CHECK SYGNO.						Frewen	NO	NO	NO		ACCESSION N
18 SUBJECT TERMS (Continue on reverse if necessary and identify by block number) 18 ABSTRACT (Continue on reverse if necessary and identify by block number) 19 ABSTRACT (Continue on reverse if necessary and identify by block number) 19 ABSTRACT (Continue on reverse if necessary and identify by block number) 19 ABSTRACT (Continue on reverse if necessary and identify by block number) 19 ABSTRACT (Continue on reverse if necessary and identify by block number) 19 ABSTRACT (Continue on reverse if necessary and identify by block number) 19 ABSTRACT (Continue on reverse if necessary and identify by block number) 19 ABSTRACT (Continue on reverse if necessary and identify by block number) 19 ABSTRACT (Continue on reverse if necessary and identify by block number) 19 ABSTRACT (Continue on reverse if necessary and identify by block number) 19 ABSTRACT (Continue on reverse if necessary and identify by block number) 19 ABSTRACT (Continue on reverse if necessary and identify by block number) 19 ABSTRACT (Continue on reverse if necessary and identify by block number) 19 ABSTRACT (Continue on reverse if necessary and identify by block number) 19 ABSTRACT (Continue on reverse if necessary and identify by block number) 19 ABSTRACT (Continue on reverse if necessary and identify by block number) 19 ABSTRACT (Continue on reverse if necessary and identify by block number) 19 ABSTRACT (Continue on reverse if necessary and identify by block number) 19 ABSTRACT (Continue on reverse if necessary and identify by block number) 19 ABSTRACT (Continue on reverse if necessary and identify by block number) 19 ABSTRACT (CONTINUE ON AND ABSTRACT (CONTINUE ON ABSTRACT (CONTINUE ON AND ABSTRACT (CO	immunodet Exposure 12 PERSONAL	ection of to T-2 To AUTHOR(S)	T-2 I xin	Metaboli	-				or Intr	amuscu	lar
16 SUPPLEMENTARY NOTATION 17 COSATI CODES 18 SUBJECT TERMS (Continue on reverse if necessary and identify by block number). 19 ABSTRACT (Continue on reverse if necessary and identify by block number). 19 ABSTRACT (Continue on reverse if necessary and identify by block number). 19 ABSTRACT (Continue on reverse if necessary and identify by block number). 19 ABSTRACT (Continue on reverse if necessary and identify by block number). 19 ABSTRACT (Continue on reverse if necessary and identify by block number). 19 ABSTRACT (Continue on reverse if necessary and identify by block number). 19 ABSTRACT (Continue on reverse if necessary and identify by block number). 19 ABSTRACT (Continue on reverse if necessary and identify by block number). 19 ABSTRACT (Continue on reverse if necessary and identify by block number). 19 ABSTRACT (Continue on reverse if necessary and identify by block number). 19 ABSTRACT (Continue on reverse if necessary and identify by block number). 19 ABSTRACT (Continue on reverse if necessary and identify by block number). 21 ABSTRACT (Continue on reverse if necessary and identify by block number). 22 ABSTRACT (Continue on reverse if necessary and identify by block number). 23 ABSTRACT (Continue on reverse if necessary and identify by block number). 24 ABSTRACT (Continue on reverse if necessary and identify by block number). 25 AUDITION (ABSTRACT SECONITY CLASSIFICATION (CLASSIFICATION)) 26 ABSTRACT (Continue on reverse if necessary and identify by block number). 27 ABSTRACT (Continue on reverse if necessary and identify by block number). 28 ABSTRACT (Continue on reverse if necessary and identify by block number). 29 DISTRIBUTION (ABSTRACT SECONITY CLASSIFICATION) 20 DISTRIBUTION (ABSTRACT SECONITY CLASSIFICATION) 20 DISTRIBUTION (ABSTRACT SECONITY CLASSIFICATION) 21 ABSTRACT (CONTINUE INDIVIDUAL (ABSTRACT SECONITY CLASSIFICATION)							L		Oarl 116	DACK (DUAT :
16 SUBJECT TERMS (Continue on reverse if necessary and identify by block number). FIELD GROUP SUBGROUP T-2, T-2 metabolites, ELISA, RIA, oral, dermal 19 ABSTRACT (Continue on reverse if necessary and identify by block number) T-2 toxin inetabolites were detected in rat urines up to 2 weeks after the animals' exposur to sublethal dose of T-2 toxin. Urines were assayed for hT-2 or T-2 tetraol by / radioinmumoassay (RIA) with polyclonal antibody. Sensitivity of the RIA was 5 n) all for hT-2 and 50 my/ml for T-2 tetraol. Sume urines were assayed in parallel with an enzymentiaked immunoscribent assay (ELISA) developed for T-2 with a monoclonal antibody that cross reacts with hT-2. The sensitivity of the ELISA for detection of hT-2 in rat urines was 25 my/ml. Orines were diluted high tackgrounds, which appeared as a rise positives in the T-2 tetraol RIA and ELISA assays. T-2 or its metabolites could be detected up to 96 hrs after an imminjection of T-2 toxin. In rate exposed of ally intermally, metabolites were detected up to 10 or 16 days, respectively. "T-2 equivalents" detected by the ELISA correlated with the HT-2 or hT-2-like campounds detected by RIA. 10 DISTRIBUTION/AVAILABILITY OF ABSTRACT DEFINITION (LASSERCATION) T-2 NAME OF RESPONSIBLE INDIVIDUAL. 21 ABSTRACT RECORD CLASSERCATION		,				25 Ju	ly 19	88	. 50,7		
FIELD GROUP SUB-GROUP 1-2, T-2 metabolites, ELISA, RIA, oral, dermal 1-2 hastract (Continue on reverse if recessary and identify by block number) 1-2 toxin metabolites were detected in rat urines up to 2 weeks after the animals' exposur to sublethal dose of T-2 toxin. Orines were assayed for HT-2 or T-2 tetraol by / radioimmunoassay (RIA) with polyclonal antibody. Sensitivity of the RIA was 5 n) all for HT-2 and 50 ng/ml for T-2 tetraol. Some urines were assayed in parallel with an enzymenthal immunoscribent assay (ELISA) developed for T-2 with a monoclonal antibody that cross reacts with HT-2. The sensitivity of the ELISA for detection of HT-2 in rat urines was 25 mg/ml. Orines were diluted 1:2 or 1:5 to eliminate high backgrounds, which appeared as a rise positives in the T-2 tetraol RIA and ELISA assays. T-2 or its metabolites could be detected up to 96 hrs after an im injection of T-2 toxin. In rate exposed orally for termally, metabolites were detected up to 10 or 16 days, respectively. "T-2 equivalents" detected by the ELISA correlated with the HT-2 or HT-2-like compounds detected by RIA. 10 DETERBOTION/AVARIABILITY OF ABSTRACT Determined as a series of the HT-2 or HT-2-like compounds detected by RIA.	16 SUPPLEME	NTARY NOTA	TION ,								
T-2, T-2 metabolites, ELISA, RIA, oral, dermal 19 ABSTRACT (Continue on reverse if necessary and identify by block number) T-2 toxin metabolites were detected in rat urines up to 2 weeks after the animals' exposur to sublethal dose of T-2 toxin. Urines were assayed for BT-2 or T-2 tetraol by / radioimmunoassay (RIA) with polyclonal antibody. Sensitivity of the RIA was 5 n) all for BT-2 and 50 ng/ml for T-2 tetraol. Some urines were assayed in parallel with an enzyme-11mked immunoscribent assay (ELISA) developed for T-2 with a monoclonal antibody that cross reacts with BT-2. The sensitivity of the ELISA for detection of BT-2 in rat urines was 25 m/ml. Orines were diluted li2 or 1:5 to eliminate high backgrounds, which appeared as also positives in the T-2 tetraol RIA and ELISA assays. T-2 or its metabolites could be betected up to 96 hrs after an im injection of T-2 toxin. In rats exposed orally in termally, metabolites were detected up to 10 or 16 days, respectively. "T-2 equivalents" detected by the ELISA correlated with the HT-2 or HT-2-like compounds detected by RIA. 10 DISTRIBUTION/AVARIABILITY OF ABSTRACT DISTRIBUTION DISTRIBUTION DISTRIBUTION DISTRIBUTION DISTRIBUTION DISTRIBUTION DISTRIBUTION DISTRIBUTION DISTRIBUTION	17	COSATI	CODES		18 SUBJECT TERMS	(Continue o	n reverse	if necessary an	id identify	by block	number).
19 ABSTRACT (Continue on reverse if necessary and identify by block number) T-2 toxin inetabolites were detected in rat urines up to 2 weeks after the animals' exposur to sublethal dose of T-2 toxin. Urines were assayed for HT-2 or T-2 tetraol by / radioimmunoassay (RIA) with polyclonal antibody. Sensitivity of the RIA was 5 n) all for HT-2 and 50 ng/ml for T-2 tetraol. Some urines were assayed in parallel with an enzymentinked immunoascribent assay (ELISA) developed for T-2 with a monoclonal antibody that cross reacts with HT-2. The sensitivity of the ELISA for detection of HT-2 in rat urines was 25 ng/ml. Onlines were diluted 1:2 or 1:5 to eliminate high backgrounds, which appeared as raise positives in the T-2 tetraol RIA and ELISA assays. T-2 or its metabolites could be detected up to 96 hrs after an iminjection of T-2 toxin. In rats exposed ofally for termally, metabolites were detected up to 10 or 16 days, respectively. "T-2 equivalents" detected by the ELISA correlated with the HT-2 or HT-2-like exampounds detected by RIA. 10 DISTRIBUTION/AVARIABILITY OF ABSTRACT	FIELD	GROUP	SU	-GROUP]						
T-2 toxin metabolites were detected in rat urines up to 2 weeks after the animals' exposur to sublethal close of T-2 toxin. Orines were assayed for HT-2 or T-2 tetracility / radioimmunoassay (RIA) with polyclonal antibody. Sensitivity of the RIA was 5 m) all for HT-2 and 50 mg/ml for T-2 tetracil. Some urines were assayed in parallel with an enzymetimed immunoscribent assay (ELISA) developed for T-2 with a monoclonal antibody that cross reacts with HT-2. The sensitivity of the ELISA for detection of HT-2 in rat urines was 25 mg/ml. Orines were diluted 1:2 or 1:5 to eliminate high backgrounds, which appeared as reactive in the T-2 tetracil RIA and ELISA assays. T-2 or its metabolites could be detected up to 96 hrs after an im injection of T-2 toxin. In rule exposed of ally in termally, metabolites were detected up to 10 or 16 days, respectively. MT-2 equivalents detected by the ELISA correlated with the HT-2 or HT-2-like compounds detected by RIA. 10 DISTRIBUTION/AVARABILITY OF ABSTRACT OF HT-2 or HT-2-like compounds detected by RIA.			ļ		1-2, T-2 meta	mottes,	51.15	A, RIA, Or	ai, Ger	nu I	
T-2 toxin metabolites were detected in rat urines up to 2 weeks after the animals' exposur to sublethal close of T-2 toxin. Orines were assayed for HT-2 or T-2 tetracility / radioimmunoassay (RIA) with polyclonal antibody. Sensitivity of the RIA was 5 m) all for HT-2 and 50 mg/ml for T-2 tetracil. Some urines were assayed in parallel with an enzymetimed immunoscribent assay (ELISA) developed for T-2 with a monoclonal antibody that cross reacts with HT-2. The sensitivity of the ELISA for detection of HT-2 in rat urines was 25 mg/ml. Orines were diluted 1:2 or 1:5 to eliminate high backgrounds, which appeared as reactive in the T-2 tetracil RIA and ELISA assays. T-2 or its metabolites could be detected up to 96 hrs after an im injection of T-2 toxin. In rule exposed of ally in termally, metabolites were detected up to 10 or 16 days, respectively. MT-2 equivalents detected by the ELISA correlated with the HT-2 or HT-2-like compounds detected by RIA. 10 DISTRIBUTION/AVARABILITY OF ABSTRACT OF HT-2 or HT-2-like compounds detected by RIA.	9 ABSTRACT	(Continue on	/#V#/30	if necessary	and identify by block	number)					
radioinmunoassay (RIA) with polyclonal antibody. Sensitivity of the RIA was 5 n) all for iHT-2 and 50 ng/ml for T-2 tetracl. Some urines were assayed in parallel with an enzymentimed immunoascreent assay (ELISA) developed for T-2 with a monoclonal antibody that cross reacts with HT-2. The sensitivity of the ELISA for detection of HT-2 in rat urines was 25 mg/ml. Orines were diluted 1:2 or 1:5 to eliminate high backgrounds, which appeared as 1 dise positives in the T-2 tetracl RIA and ELISA assays. T-2 or its metabolites could be detected up to 96 hrs after an imminjection of T-2 tokin. In rats exposed orally by detected up to 96 hrs after an imminjection of T-2 tokin. In rats exposed orally by detected by the ELISA correlated with the HT-2 or HT-2-like compounds detected by RIA. **CONTRIBUTION/AVABLABILITY OF ABSTRACT** **CONTRI											•
radioinmumoassay (RIA) with polyclonal antibody. Sensitivity of the RIA was 5 m) all for HT-2 and 50 mg/ml for T-2 tetradl. Some urines were assayed in parallel with an enzyme-linked immumoscreent assay (ELISA) developed for T-2 with a monoclonal antibody that cross reacts with HT-2. The sensitivity of the ELISA for detection of HT-2 in rat urines was 25 mj/ml. Orines were diluted 1:2 or 1:5 to eliminate high backgrounds, which appears as a dise positives in the T-2 tetradl RIA and ELISA assays. T-2 or its metabolites could be detected up to 96 hrs after an im injection of T-2 toxin. In rats exposed orally of termally, metabolites were detected up to 10 or 16 days, respectively. "T-2 equivalents" detected by the ELISA correlated with the HT-2 or HT-2-like compounds detected by RIA. O DISTRIBUTION/AVAILABILITY OF ABSTRACT O DIC USERS											exposura
INFO DISTRIBUTION/AVARABILITY OF ABSTRACT ODER TRACE											al tor
reacts with HT-2. The sensitivity of the ELISA for detection of HT-2 in rat urines was 25 skj/ml. Orines were diluted li2 or li5 to eliminate high backgrounds, which appeared as rilse positives in the T-2 tetraol RIA and ELISA assays. T-2 or its metabolites could be detected up to 96 hrs after an im injection of T-2 toxin. In rats exposed orally of termally, metabolites were detected up to 10 or 16 days, respectively. "T-2 equivalents" detected by the ELISA correlated with the HT-2 or HT-2-like compounds detected by RIA. O DISTRIBUTION/AVAILABILITY OF ABSTRACT CHOICE SYMBOLIC HAME OF RESPONSIBLE INDIVIOUAL 21 ABSTRACT SECURITY CLASSIFICATION 22 SELEPHONE (INClude Area Code) 22c OFFICE SYMBOLIC HAME OF RESPONSIBLE INDIVIOUAL		•		•	•			•		,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,	
The positives in the T-2 tetraol RIA and ELISA assays. T-2 or its metabolites could be detected up to 96 hrs after an im injection of T-2 toxin. In ruts exposed orally of dermally, metabolites were detected up to 10 or 16 days, respectively. "T-2 equivalents" detected by the ELISA correlated with the HT-2 or HT-2-like exampounds detected by RIA. O DISTRIBUTION/AVAILABILITY OF ABSTRACT O DISTRIBUTION/AVAILABILITY OF ABSTRACT O DISTRIBUTION/AVAILABILITY OF ABSTRACT O DISTRIBUTION/AVAILABILITY OF ABSTRACT O DISTRIBUTION MITEO SAME AS HET O DISTRIBUTION (INCIDUM AREA COME) 22c OFFICE SYMBO).	linked in	munoscrbe	nt as:	say (ELI	SA) developed f	or T-2	vith a	monoclona	l antib	ody th	at cross
I also positives in the T-2 tetraol RIA and ELISA assays. T-2 or its metabolites could be detected up to 96 hrs after an im injection of T-2 toxin. In ruts exposed orally of dermally, metabolites were detected up to 10 or 16 days, respectively. "T-2 equivalents" detected by the ELISA correlated with the HT-2 or HT-2-like exampounds detected by RIA. O DISTRIBUTION/AVAILABILITY OF ABSTRACT CHOICLASSIFIED THE MITTER CONTINUE OF A SAME AS HET COUNTY DESIRED THE CONTINUE OF THE SAME AS HET CONTINUE OF THE SECOND CONTINUE OF THE SAME AS HET CONTINUE OF THE SECOND CONTINUE OF THE SAME OF THE											
detected up to 96 hrs after an im injection of T-2 toxin. In rats exposed orally or termally, metabolites were detected up to 10 or 16 days, respectively. "T-2 equivalents" detected by the ELISA correlated with the HT-2 or HT-2-like composites detected by RIA. O DISTRIBUTION/AVAILABILITY OF ABSTRACT CHOICLASSIFIEDAING MITEO COMME AS HET CORRESPONSIBLE INDIVIDUAL 21 ABSTRACT SECURITY CLASSIFICATION 22 TELEPHONE (INCIDENT AREA CORRESPONSIBLE INDIVIDUAL)											
dermally, metabolites were detected up to 10 or 16 days, respectively. "T-2 equivalents" detected by the ELISA correlated with the HT-2 or HT-2-like examposinds detected by RIA. 30 DISTRIBUTION/AVAILABILITY OF ABSTRACT SAME AS RET DETECTION 21 ABSTRACT SECURITY CLASSIFICATION 22 ABSTRACT SECURITY CLASSIFICATION 23 ABSTRACT SECURITY CLASSIFICATION 24 ABSTRACT SECURITY CLASSIFICATION 25 TELEPHONE (INCIDENT AREA COME) 22C OFFICE SYMBOL											
O DISTRIBUTION/AVAILABILITY OF ABSTRACT O UNICLASSIFIED UNION/MITED SAME AS RET OFFICE SYMBOL AND NAME OF RESPONSIBLE INDIVIOUAL 21 ABSTRACT SECURITY CLASSIFICATION 22 ABSTRACT SECURITY CLASSIFICATION 23 ABSTRACT SECURITY CLASSIFICATION 24 ABSTRACT SECURITY CLASSIFICATION 25 TELEPHONE (INCIDENT AREA CORE) 22C OFFICE SYMBOL	termally,	metaboli	tus w	ere dete	cted up to 10 c	or 16 day	/s, 🖂	spectively	. "T-2	oquiv	alents"
21 ABSTRACT SECURITY CLASSIFICATION O DISTRIBUTION / AVAILABILITY OF ABSTRACT O DISTRIBUTION / AVAILABILITY											
DENCLASSIFIED TINE MITTED SAME AS HET DETCHSERS 226 TELEPHONE (INCIDENT ARE CORE) 220 OFFICE SYNTHETE.	. 4*			•				•	🎉 mir i samen 🕳	1	
DENCLASSIFIEDANCIMITED DEAME AS AFT DOTIC USERS 24 NAME OF RESPONSIBLE INDIVIDUAL 226 TELEPHONE (INCIUDE Area Code) 226 OFFICE SYNERO.	o ostabut	ION/AVAILABI	LITY OF	ABSTRACT	<u> </u>	21 ABST	ACT SEC	URITY CLASSIFIC	Ation	·	- der Vije is Miller der der der der der der der der der d
	CONCLASS	SIFIED TINE MIT	EO 🗆	SAME AS H	IFT 🗍 OTH OSERS						
D form 1473. JUN 86	A NAME OF	RESPUNSIBLE	CIVION	UAL		Zab Tere	HOME (H	woude Area Cod	el 22c of	FICE SYS	tuc).
THE A SECURE STORY OF THE PRODUCTION ACCURAGE AND ADMINISTRAL STREET, AND ASSOCIATION ASSO	D form 147	3 HIM SE				1					Made Private Systems

IMMUNODETECTION OF T-2 METABOLITES IN PAT URINES AFTER DERMAL, ORAL, OR INTRAMUSCULAR EXPOSURE TO T-2 TOXIN

JOHN F. HEWETSON*, ROBERT W. WANNEMACHER, Jr.*,
AND ROBERT J. HAWLEY*

*United States Army Medical Research Institute of Infectious Diseases, and †United States Army Medical Materiel Development Activity, Fort Detrick, Frederick, MD 21701-5011

Running Title: T 2 METABOLITES IN RAT URINES

Corresponding Author:

John F. Hewetson

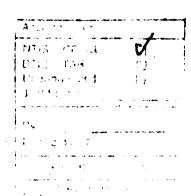
Pathophysiology Division

USAMRIID, Ft. Detrick

Frederick, MD 21701-5001

(301)663-7181

1





A-1

Immunodetection of T-2 Metabolites in Rat Urines after Dermal. Oral. or Intramuscular Exposure to T-2 Toxin. Hewetson, J.F., Wannemacher, R.W. Jr., and Hawley, R.J. (1988). Fundam. Appl. Toxicol. --, -- --. T-2 toxin metabolites were detected in rat urines up to 2 weeks after the animals' exposure to sublethal dose of T-2 toxin. Urines were assayed for HT-2 or T-2 tetraol by radioimmunoassay (RIA) with polyclonal antibody. Sensitivity of the RIA was 5 ng/ml for HT-2 and 50 ng/ml for T-2 tetraol. Some urines were assayed in parallel with an enzyme-linked immunosorbent assay (ELISA) developed for T-2 with a monoclonal antibody that cross reacts with HT-2. The sensitivity of the ELISA for detection of HT-2 in rat urines was 250 ng/ml. Urines were diluted 1:2 or 1:5 to eliminate high backgrounds, which appeared as false positives in the T-2 tetraol RIA and ELISA assays. T-2 or its metabolites could be detected up to 96 hr after an im injection of T-2 toxin. In rats exposed orally or dermally, metabolites were detected up to 10 or 16 days, respectively."T-2 equivalents" detected by the ELISA correlated with the HT-2- or HT-2-like compounds detected by RIA.

The presence of T-2 toxin in contaminated grain (Chang, et al. 1974: Ellison and Kotsonis 1973; Hsu, et al. 1972; Jemmali, et al. 1978; Mirocha, et al. 1979; Romer, et al. 1978) and its reported use as a biological warfare weapon in Southeast Asia and Afghanistan (Mirocha, et al. 1983; Watson, et al. 1984) has generated many studies on the detection of T-2 by mass spectrometry (Mirocha, et al. 1983), high pressure liquid chromatography (Stack and Eppley 1980) thin layer chromatography (Pace 1986; Takeda 1979), bioassay (Sukroongreung 1984; Sukroongreung, et al. 1984a; Chung, et al. 1974) and immunoassay techniques (Fan, et al. 1984; Fontelo, et al. 1983; Chu 1986; Pestka, et al. 1981; Peters, et al. 1982). Studies carried out with tritiated toxin show that T-2 is rapidly metabolized to HT-2 and T-2 tetraol and the parent compound is not present in urines of exposed guinea pigs (Pace, et al. 1985; Pace, et al. 1985a) and monkeys (Mereish, et al. 1987). Radio-labeled toxins are appropriate for laboratory studies, but not useful for evaluation of natural exposure. Several conventional immunoassay techniques based on tritiated ligands (Fontelo, et al. 1983; Lee and Chu 1981, 1981a) and enzymes conjugated to ligands or antibodies (Fan, et al. 1984; Gendloff, et al. 1984; Pestka, et al. 1981; Peters, et al. 1982) have formed the basis of most of the efforts for rapid detection both in environmental samples and biological fluids. Cross reactivities occur and are exploited to broaden the number of substances that a given assay can detect. Therefore, each assay must be standardized for a particular substance and all known cross reactivities investigated before it can be stated with certainty what is present in an unknown sample. The previous studies have used spiked samples of urine or blood to demonstrate the feasibility of such procedures and often have had problems with background and non-specific reactions (Fan, et al. 1984; Lee and Chu 1981). In order to demonstrate the practical feasibility of confirming an environmental exposure to T-2 toxin, we evaluated two assays for the detection of T-2 metabolites in rat urines. These studies were compatible with an evaluation of accidental or intentional environmental exposure. Three routes of exposure were used and the kinetics of excretion evaluated.

∂2.€/55/5584 € 62/62/24€ 62/55/3/€ **222266/6/26/3/€ 62/26/3/€ 22/2**6/2<mark>6/26/3</mark>

METHODS

Procession to a second a possession person

TO COLORS OF BOOK OF THE B

Toxin. T-2 toxin was obtained from Myco Labs, Inc., Chesterfield, Missouri.

Polyclonal antiserum. Antibodies to HT-2 were raised by conjugating the molecule to bovine serum albumin and immunizing rabbits or goats (Fan. et al. 1987; Chu 1986). Antibodies to T-2 tetraol were produced in rabbits by first conjugating T-2 tetraol to sheep gamma globulin. The resulting antibodies were titrated against the homologous labeled ligand for optimal reactivity in an RIA. HT-2 antisera were supplied by Dr. Chu, University of Wisconsin (Fan. et al. 1987) and T-2 tetraol antisera were produced and supplied by Bio-Metric Systems, Inc., Eden Prairie, Minnesota. ¹

Monoclonal antibody. A monoclonal antibody to T-2 (15H6) was originally developed by Dr. Ken Hunter at the Uniformed Services University of the Health Sciences, Bethesda, Maryland. (Hunter, et al. 1985). Quantities of this monoclone as IgG-purified ascites fluid were produced by Charles River Biotechnical Service, Inc. Wilmington, Massachusetts. The antibody was titrated in the RIA against homologous ligand for optimal reactivity.

Radioimmunoassay. A standard RIA was used as previously described (Fontelo, et al. 1983) with slight modifications. 100 μ l of sample, 50 μ l of ³H-ligand (20,000 dpm), and 50 μ l of diluted antibody were combined and incubated at room temperature for 1 hr. Ammonium sulfate (250 μ l, 76%) was added, the tubes incubated for 1 hr, and centrifuged at 3000 rpm in an International Model PR6 centrifuge. Supernatants were counted for radioactivity and the amount of toxin present in the samples determined from standard curves run in phosphate buffered saline (PBS). Normal urines were included in all assays as a control for nonspecific binding.

Dr. Chu and Bio-Metric Systems are under contract with the U. S. Army.

Enzyme-Linked Immunosorbent assay. An ELISA test kit selected as the best prototype for detection of T-2 in environmental and biological samples, consisted of a standard competitive ELISA with T-2 adsorbed onto the surface of plastic plates. Unknown sample plus monoclonal T-2 antibody conjugated to horseradish peroxidase were added to the wells and incubated. After 2 hr, wells were washed and 2,2'Azinobis(3- ethylbenz-thiazoline sulfonic acid) (ABTS) substrate added. The absence of color indicated a positive reaction, that is, presence of toxin. The results were read as a + or - by comparison to controls included in each assay.

Animals. Fisher F344 rats (250-400g) from Charles River, Wilmington, Massachusetts, were used for all studies. Rats were housed in plastic metabolic cages (Nalgene, Inc. Rochester, N.Y.) with free access to food and water. Urine and feces were collected daily and stored at 4°C.

Experimental design. Three groups of rats were exposed to T-2 toxin either im, orally, or dermally. Group 1 received 0.5 mg/kg, im in PBS by injection in the thigh muscle. Group 2 was fasted 24 hr before receiving an oral dose of 2 mg/kg T-2 in propylene glycol by inoculation with a 23-gauge, 3-in. intubation needle attached to a 1 ml syringe. Group 3 animals were shaved with electric clippers and 24 hr later, 0.1 ml of the toxin (0.5 or 1.0 mg/kg) in dimethylsulfoxide was applied to a 4 x 4 cm2 area on the shaved skin. A jacketed barrier was applied over the application site, as described previously (Wannemacher, et al. 1985). Controls of carrier only were included in all groups.

RESULTS

Titration of Antisera

Both polyclonal sera and 15H6 monoclonal anti-sera had good reactivity against the homologous ligand in the RIA (data not shown). Based on 50% binding, dilution of the antisera selected for further studies was 1:25, 1:20, and 1:35 for T-2, HT-2 and T-2 tetraol, respectively. Cross reactivities with related mycotoxins were determined to show the specificity of each antiserum. Anti-T-2 showed complete cross reactivity with HT-2 as also snown by Hunter (Hunter, et al. 1985), and no cross reactivity with T-2 tetraol. Anti-T-2 was 30% cross reactive with T-2 and unreactive with T-2 tetraol. Anti-T-2 tetraol did not cross react with T-2, but was 30% cross reactive with HT-2.

False Positive Reactions and Toxin Detection Limits

Previous studies showed that individual normal urines exhibited false positive reactions in the T-2 tetraol RIA (Hewetson, unpublished). The false positive reaction was eliminated by diluting the urine 1:2. Therefore, the detection limit of T-2 tetraol in urine was defined at a 1:2 dilution as the bound/free (B/F) value that was greater than 2 standard errors from the B/F value of a group of 20 control urines diluted 1:2. The same definition applied to HT-2 detection, except that undiluted urines were assayed with no false positive reactions. Detection limits for each of the toxins was 5 ng/ml for HT-2 and 50 ng/ml for T-2 tetraol.

Standardization of ELISA Kit

Since the monoclonal antibody against T-2 cross reacts with T-2 (Hunter, et al. 1985, Hewetson, unpub'ished), we included HT-2 in our evaluation. The ELISA kit was capable of detecting of both T-2 and HT-2 in buffer at

concentrations of 25 ng/ml and 50 ng/ml, respectively (Table 1). The ELISA kit also produced sporadic false-positive reactions with control urine. A 1:5 dilution of the urine was necessary to eliminate this problem, making the ELISA detection limit of HT-2 in urines about 250 ng/ml.

T-2 Metabolite Detection in Urine by RIA

A series of studies were designed to evaluate the patterns of HT-2 and T-2 tetraol excretion in urines of exposed rats. Table 2 presents the results obtained after their im exposure. T-2 tetraol and trace amounts of HT-2 were detected up to day 3 in rat 1. Since this rat did not produce urine on day 1, total toxin excreted was similar to that excreted by rats 2 and 3.

T-2 tetraol and HT-2 excretion after oral exposure occurred up to 9 days after exposure (Fig. 1 and 2). Dermal exposure to T-2 showed an excretion pattern that extended beyond that observed for the other two routes of exposure (Fig. 3 and 4). T-2 toxin metabolites, HT-2 and T-2 tetraol, were still detected up to 16 days after exposure.

T-2 Metabolite Detection in Urine by ELISA

Selected urine samples from the oral-exposure series were tested in the ELISA kit. All urines were not evaluated by this method because of a limitation on the number of tests available. Table 3 presents a comparison between the two methods for detection of HT-2 (RIA) and "T-2 equivalents" (ELISA). Urine samples containing HT-2 >250 ng/ml as measured by RIA were positive by the ELISA. Lower amounts of HT-2 could not be detected by the ELISA.

DISCUSSION

These studies describe the excretion patterns of T-2 metabolites in urine after three routes of exposure. We also compared detection results obtained with an RIA specific for HT-2 to results from a commercially available kit for detecting "T-2 equivalents." Previous studies show that the principal metabolites of T-2 are HT-2 and T-2 tetraol (Pace, et al. 1985; Pace, et al. 1985a; Corley, et al. 1985; Yoshizawa, et al. 1980). Therefore an assay for these urinary metabolites should provide evidence of T-2 exposure. Pace, et al (1985a) reported that in guinea pigs exposed im to ³H T-2, radioactivity in urlne peaked at 24 hr and rapidly decreased over the next 4 days. Matsumoto, et al. (1978) reported rapid excretion of radioactivity after oral administration of ³H T-2 Our results also showed rapid excretion of T-2 metabolites after im exposure as detected by the RIA. After oral administration of T-2, the majority of metabolites were excreted by day 4 (Fig. 3 and 4), but there was a low, detectable amount of HT-2 up to day 8 in all rats. Low levels of T-2 tetraol were detected up to day 10 in two rats studied. Matsumoto, et al. (1978) reported most of the radioactivity was excreted by 72 hr in two rats orally dosed with T-2. Most of the HT-2 and T-2 tetraol excreted from rats dosed dermally with T-2 was complete by day 10. However, low levels of both metabolites were detected up to day 16 after exposure. This was most likely due to the slow absorption of T-2 from the skin.

As HT-2 showed complete cross reactivity with the anti-T-2 monoclonal antibody (Hunter, et al. 1985), we evaluated exposure to T-2 by testing urines with an ELISA kit designed for T-2 testing. These results compared favorably with results obtained with the RIA specific for HT-2.

False-positive reactions were not a problem with HT-2 detection in the RIA, even in undiluted urine. Sensitivity was similar to that of HT-2 in buffer, or about 5 ng/ml. T-2 tetraol detection by RIA and HT-2 detection by ELISA presented significant interference. These false-positive reactions were eliminated by diluting the urines to a point where no false-positives

occurred in a battery of control urines. Although this simple solution does not provide an answer to the identification of the interfering material, it offers a practical alternative. By diluting samples 1:5 for the ELISA, practical detection limits for HT-2 in undiluted urine were raised to about 250 ng/ml. The RIA can detect HT-2 at lower levels than the ELISA (Table 3), but the 250 ng/ml level for the ELISA was more than adequate for routine detection in urines up to several days after exposure to a sublethal dose of T-2.

These studies support Mirocha's observation (Mirocha, et al. 1983) in human samples from Southeast Asia that HT-2 could be detected 2 weeks after exposure. Although we document a useful assay for detection of sublithal exposure to T-2 by an oral or dermal route, incorporation of either anti-HT-2 or anti-T-2 tetraol antibodies into the kit should provide greater sensitivity for detection of T-2 metabolites.

REFERENCES

CHU, F.S. 1986. Immunoassays for mycotoxins. p. 207-237. Modern methods in the analysis and structural elucidation of mycotoxins. Academic Press, Inc., New York, N.Y.

CHUNG, C.W., TRUCKSESS, M.W., GILES, A.L. Jr, and FRIEDMAN, L. 1974. Rabbit skin test for estimation of T-2 toxin and other skin in itating toxins in contaminated corn. J. Assoc. Off. Anal. Chem. 57, 1121-1127.

CORLEY, R.A., SWANSON, S.P., and BUCK, W.B. 1985. Glucuronide conjugates of T-2 toxin and metabolites in swine bile and urine. *J. Agric. Food Chem.* **33**, 1085-1089.

ELLISON, R.A. and KOTSONIS, F.N. 1973. T-2 toxin as an emetic factor in meldy com. *Appl. Microbiol.* **26**, 540-543.

FAN, T.S., ZHANG, G.S. and CHU, F.S. 1984. An indirect enzyme-linked immunosorbent assay for T-2 toxin in biological fluids. *J. Food Prot.* 47, 964-967.

FAN, T.S., ZHANG, G.S., and CHU, F.S. 1987. Production and characterization of antibodies against HT-2 toxin and T-2 tetraol tetraacetate. *Appl. Environ. Microbiol.* **53**, 17-21.

FONTELO, P.A., BEHELER, J., BUNNER, D.L., and CHU, F.S. 1933. Detection of T-2 toxin by an improved radioimmunoassay. *Appl. Environ. Microbiol.* **45**, 640-643.

GENDLOFF, E.H., PESTKA J.J., SWANSON, S.P., and HART, L.P. 1984. Detection of T-2 toxin in *Fusarium sporotrichioides*-infected corn by enzyme-linked immunosorbent assay. *Appl. Environ. Microbiol.* 47, 1161-1163.

HSU, I. C., SMALLEY, E.B., STRONG, F.M., and RIBELIN, W.E. 1972. Identification of T-2 toxin in moldy corn associated with a lethal toxicosis in dairy cattle. *Appl. Microbiol.* **24**, 684-690.

HUNTER, K.W. Jr., BRIMFIELD, A.A. MILLER, M., FINKELMAN, F.D., and CHU, S.F. 1985. Preparation and characterization of monoclonal antibodies to the trichothecene mycotoxin T-2. *Appl. Environ. Microbiol.* **49**, 168-172.

JEMMALI, M., UENO, Y., ISHII, K., FRAYSSINET, C., and ETIENNE, M. 1978. Natural occurrence of trichothecenes (nivalenol, deoxynivalenol, T-2) and zearalenone in corn. *Experientia* **34**, 1333-1334.

LEE, S. and CHU, F.S. 1981. Radioimmunoassay of T-2 toxin in biological fluids. *J. Assoc. Off. Anal. Chem.* **64**, 684-688.

LEE, S. and CHU, F.S. 1981a. Radioimmunoassay of T-2 toxin in corn and wheat. J. Assoc. Off. Anal. Chem. 64, 156-161.

MATSUMOTO, H., ITO, T., and UENO, Y. 1978. Toxicological approaches to the metabolites of Fusaria. XII. Fate and distribution of T-2 toxin in mice. *Jpn. J. Exp. Med.* **48**, 393-399.

MEREISH, K.A., NASEEM, S.M., DINTERMAN, R.E., PACE, J.G., WANNEMACHER, R.W., and BUNNER, D.L. 1987. Pharmacokinetics of T-2 mycotoxin and its metabolites in cynomolgus monkeys. *The Pharmacologist* **29**, 88.

MIROCHA, C.J., PAWLOSKY, R.A., CHATTERJEE, K., WATSON, S., and HAYES, W. 1983. Analysis for fusarium toxins in various samples implicated in biological warfare in Southeast Asia. *J. Assoc. Off. Anal. Chem.* **66**, 1485-1499.

MIROCHA, C.J., SCHAUERHAMER, B., CHRISTENSEN, C.M., and KOMMEDAHL, T. 1979. Zearalenone, deoxynivalenol, and T-2 toxin associated with stalk rot in co.n. Appl. Environ. Microbiol. 38, 557-558.

PACE, J.G. 1986. Metabolism and clearance of T-2 mycotoxins in perfused rat livers. Fund. Appl. Toxicol. 7, 424-433.

PACE, J.G., WATTS, M.R., BURROWS, E.P., DINTERMAN, R.E., MATSON, C., HAUER, E.C., and WANNEMACHER, R.W. Jr. 1985. Fate and distribution of ³H-labeled T-2 mycotoxin in guinea pigs. *Toxicol. Appl. Pharmacol.* **80**, 377-385.

PACE, J.G., WATTS, M.R., BURROWS, E.P., and MATSON, C. 1985. Identification of metabolites of T-2 toxin in guinea pigs. *Toxicon* 23, 602.

PESTKA, J.J., LEE, S.L., LAU, H.P., and CHU, F.S. 1981. Enzyme linked immunosorbent assay for T-2 toxin. J. Am. Oll Chem. Soc. 58, 940A 944A.

PETERS, H., DIERICH, M.P., and DOSE, K. 1982. Enzyme-linked immunosorbent assay for detection of T-2 toxin. *Appe Scylers Z. Physiol. Chem.* **363**, 1437-1441.

ROMER, T.R., BOLING, T.M., and MACDONALD, J.L. 1978. Gas liquid chromatographic determination of T-2 toxin and diacetoxyscirpenol in corn and mixed feeds. J. Assoc. Off. Anal. Chem. 61, 801-808.

STACK, M.E. and EPPLEY, R.M., 1980. High pressure liquid chromatographic determination of Satratoxins G and H in cerealgrains. J. Assoc. Off. Anal. Chem. 63, 1278-1281.

SUKROONGREUNG, S. 1984. Biological screening test for trichothecenes. J. Med. Assoc. That. 67, 432–437.

F. KROONGREUNG, S., SCHAPPERT, K.T., and KHACHATOURIANS, G.G. 1984. Survey of sensitivity of twelve yeast genera toward T-2 toxin. Appl. Enutron. Microbiol. 48, 416-419.

TAKEDA, Y. ISOHATA E. AMANO R. UCHIYANA M. 1979. Simultaneous extraction and fractionation and thin layer chromatographic determination of 14 mycotoxins in grains. J. Assoc. Off. Anal. Chem. 62, 573-578.

WANNEMACHER, R.W.Jr., BUNNER, D.L., PACE, J.G., NEUFELD, H.A., BRENNECKE, L.H., and DINTERMAN, R.E. 1985. Dermal toxicity of T-2 toxin in guinea pigs, rats, and cynomolgus monkeys. p. 423-432. In Lacey, J. (ed) *Trichothecenes And Other Mycotoxins*. John Wiley & Sons, New York.

WATSON, S.A., MIROCHA, C.J., and HAYES, A.W. 1984. Analysis for trichothecenes in samples from southeast Asia associated with "yellow rain." *Fund. Appl. Toxicol.* **4**, 700-717.

YOSHIZAWA, T., SWANSON, S.P., and MIROCHA, C.J. 1980. T-2 metabolites in the excreta of broiler chickens administered ³H-labeled T-2 toxin. *Appl. Environ. Microbiol.* **39**, 1172-1177.

TABLE 1

T-2 AND HT-2 TITRATION OF STANDARD TOXIN

SAMPLES IN ELISA KIT

·			
ng/ml		T-2	HT-2
10,000		+	•
1,000		+	+
100	•	+	•
50	١		+
25		• • • • • • • • • • • • • • • • • • •	•
12	•	+/-	•
6			-
PBS		•	. •

HT-2 AND T-2 TETRAOL RECOVERED FROM RAT URINE (µg/kg)

IM EXPOSURE TO T-2

TABLE 2

RAT	24	24 HR		48 HR		72-96 HR		120 HR	
	Ta	Hb	Т	Н	Т	Н	т	Н	
1	c	•	3.841	0.911	1.614	(TR)d	0.484	(TR)	
2	4.587	1.974	1.367	(TR)	(TR)	(TR)	•	(TR)	
3	12.506	0.992	1.965	1.910	1.846	(TR)	-	-	

a T T-2 tetraol

b н - нт-2

c - Not detected

d TR Trace

TABLE 3

HT DETECTION IN RAT URINES

ORAL EXPOSURE

DAY	ELISAª	RIA (ng/ml)
1	+	2116
2	+	5533
3	+	1250
4	•	196
5.6	•	133
7.8	+	270
9	• •	
10	-	•

a Urine diluted 1:5

LEGENDS

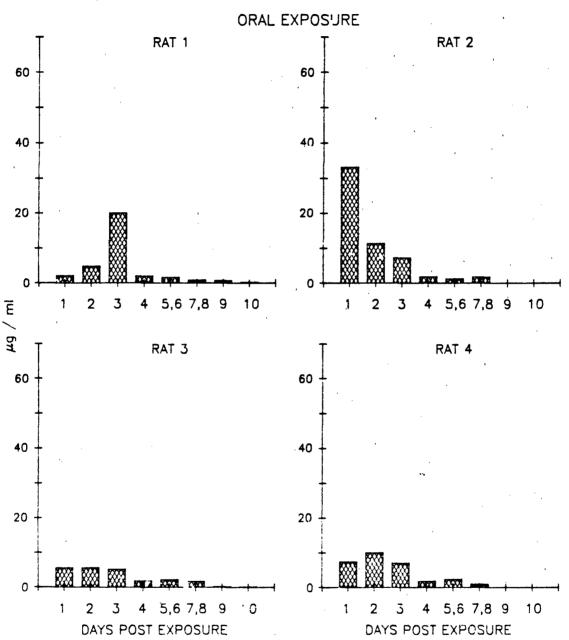
FIG 1 AND 2

Recovery of HT-2 and T-2 tetraol from individual rat urines after oral exposure to 2 mg/kg T-2 in propylene glycol. Recovery is expressed as μg toxin recovered per kg body weight.

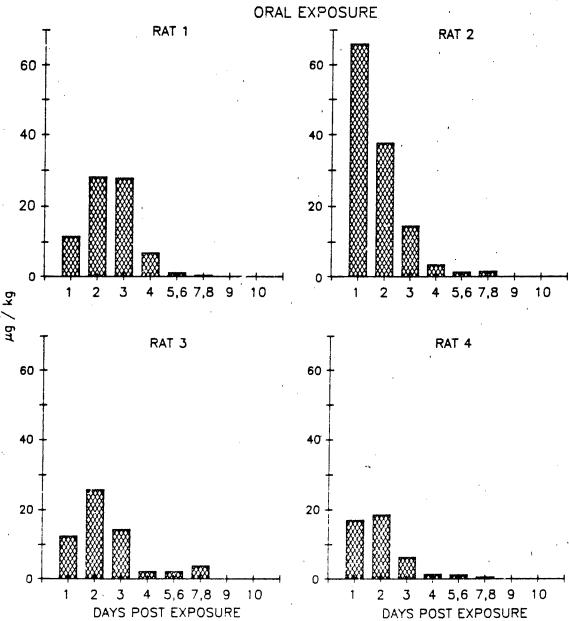
FIG 3 AND 4

Recovery of HT-2 and T-2 tetraol from rat urines after dermal exposure to T-2. Two groups of 3 rats received 1 mg/kg or 0.5 mg/kg, respectively. Recovery is expressed as µg toxin recovered per kg body weight.

TETRAOL RECOVERED FROM RAT URINES

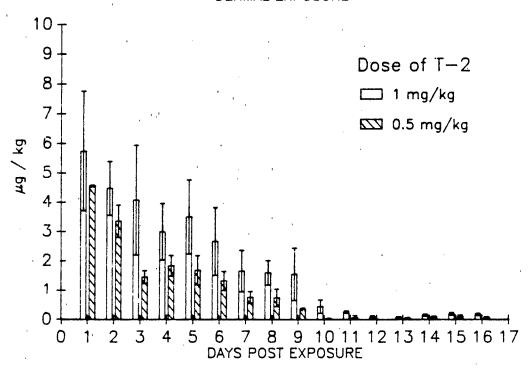


HT-2 RECOVERED FROM RAT URINES



HT-2 RECOVERED FROM RAT URINES

DERMAL EXPOSURE



TETRAOL RECOVERED FROM RAT URINES DERMAL EXPOSURE

